

SUPPLEMENTARY METHODS

Patient Sample Collection: The study was conducted with IRB approval at the University of Southern California Norris Comprehensive Cancer Center (NCCC) between January 2017 and February 2018. After obtaining informed consent, blood samples were obtained from twenty men with mCRPC encountered in the outpatient oncology clinics. For each patient, a total of three 7.5mL blood collection tubes (EDTA, Cell-Free RNA Streck, CellSave) were collected, processed, stored and transported according to each manufacturers' instructions for analysis. Specifically, the EDTA tube was mixed with 10mL LB-Fix, Cynvenio's blood stabilization solution, which was left to sit for an hour prior to shipping at ambient temperature to Cynvenio Biosystems CLIA/C AP lab for cfDNA and CTC DNA SSV analysis. Cell-free RNA Streck tubes were immediately inverted a total of 8 times prior to shipping at ambient temperature to Liquid Genomics for cfRNA relative expression analysis. CTC enumeration using the CellSave tubes was performed by the Circulating Tumor Cell/Liquid Biopsy Core at the University of Southern California (see below for more details). For a subset of patients, second time point samples were collected at treatment resistance, defined as new metastases, increased PSA, or clinical progression. Liquid biopsies were compared to tumor molecular profiles from primary tumors or metastases when available (e.g. Foundation One).

CTC enumeration by immunomagnetic enrichment: CTC enrichment and enumeration were performed on the FDA-cleared CellSearch platform (Menarini Silicon Biosystems) according to standard manufacturer's instructions. Briefly, 7.5ml blood was drawn by standard peripheral venipuncture into a CellSave preservative tube (Menarini Silicon Biosystems) and delivered at room temperature to the laboratory for processing on the same day. CellSearch enriches and identifies candidate CTCs using antibodies to epithelial-cell adhesion molecule (EpCAM) coupled with magnetic beads. After magnetic enrichment, isolated cells are stained with fluorescent nucleic acid dye (DAPI) to identify nucleated cells. Recovered cells are stained with fluorescent monoclonal antibodies to CD45 and CK 8, 18, 19 to distinguish epithelial cells from leukocytes. Cells that are EpCAM+, CD45-, CK+ and DAPI+ and fulfill morphologic criteria are counted as CTCs by a certified technician ¹.

Single CTC recovery by dielectric manipulation: CellSearch enriched patient samples were further-processed for DEPArray per manufacturer's instructions (Menarini Silicon Biosystems). Briefly, pre-coated (2% BSA) gel loading tips were used to aspirate samples from each Veridex cartridge and two additional washes were performed with SB 115 buffer (200μL) to improve recovery. Samples were then washed twice with 1000μL and 900μL of SB 115 buffer before volume reduction to 13μL for loading onto a DEPArray cartridge and into the DEPArray V2 System (Menarini Silicon Biosystems) for analysis. The DEPArray system utilizes a non-uniform electric field to exert forces on neutral, polarizable particles, such as cells, suspended in a liquid. Dielectrophoresis (DEP) is used to trap cells in dielectric cages by creating an electric field above a subset of electrodes in counter phase with the electric field of adjacent electrodes. After imaging, individual

cells of interest are gently moved with the DEP cages into holding chambers for isolation and recovery. Thus, single CTCs (CK+DAPI+CD45-) along with control single WBCs (CK-/DAPI+/CD45+) were isolated and recovered into individual MicroAmp reaction tube (Thermo Fisher). To facilitate downstream analysis, cells were pelleted (14,100xg, 30s) before a wash step was performed with 100 μ L PBS (14,100xg, 10min). Finally, volume reduction was performed by carefully aspirating the wash buffer leaving a final volume of $\sim$ 1 μ L. Samples were stored at -20C until needed as input for Ampli1 WGA kit (see below for more details).

CTC copy number variant analysis

Ampli1™ whole genome amplification, DNA library construction and whole genome sequencing: DNA of isolated cells was amplified using the Ampli1™ WGA kit according to manufacturer's instructions (Menarini Silicon Biosystems, Ampli1 QC Kit, September 2015 User Manual V1.2.WGQC4). Quality of Ampli1™ WGA products was checked using Ampli1™ QC kit (Menarini Silicon Biosystems) and only products with at least 3 amplified bands were retained. 5 μ L of Ampli1™ WGA product was transferred into a new tube and purified with 1.8X SPRIselect Beads (Beckman Coulter) according to manufacturer instructions and eluted in 12.5 μ L TE. The Ampli1™ LowPass kit, (Menarini Silicon Biosystems) was used to prepare libraries for low-pass WGS (Menarini Silicon Biosystems, Whole Genome Amplification for Single Cells, June 2015 User Manual V3.WG001U). In brief, 10–50 ng of purified primary Ampli1™ WGA product was re-amplified using hybrid PCR primers, including barcoded adaptors compatible with the Ion Torrent™ Systems on the 5' end, and primary WGA universal adaptor on the 3' end. Barcoded libraries were quantified using Qubit dsDNA HS Assay kit on a Qubit 2.0 Fluorometer (Thermo Fisher Scientific) and pooled in equimolar concentrations to obtain a concentration of $\sim$ 34ng/ μ L. Pooled libraries were size selected (300–450 bp) using E-Gel SizeSelect™ Agarose Gels, 2% on a E-Gel Agarose Gel Electrophoresis System (Thermo Fisher Scientific) according to manufacturer's instructions (Menarini Silicon Biosystems, Ampli1 QC Kit, September 2015 User Manual V1.2.WGQC4). Size selected library pool was cleaned up with 1.2X SPRIselect Beads (Beckman Coulter) according to manufacturer's instructions and quantified using Agilent High Sensitivity DNA Kit using the Agilent Bioanalyzer 2100 instrument (Agilent). Sequencing was performed using the Ion Torrent PGM™ (Thermo Fisher Scientific, Ion AMpliSeq DNA and RNA Library Preparation User Guide, Publication Number MAN0006735, Rev C.0).

Libraries from gDNAs (100ng) were prepared using Ion Xpress Plus gDNA Fragment Library preparation kit (Thermo Fisher Scientific). Briefly, samples were fragmented for 200-base-read libraries, end repaired, ligated with adaptors, nick repaired and bead purified prior to amplification of size selected (E-Gel SizeSelect™, Thermo Fisher Scientific) fragments around 250 bp long. Fragment sizes were assessed using the Bioanalyzer system and quantified using the Ion Library TaqMan® Quantitation Kit (Thermo Fisher Scientific). Pooled libraries were used for emulsion PCR amplification (200bp) using the Ion PGM™ System (Thermo Fisher Scientific).

Sequence alignment, read counting and normalization: Signal processing, base calling and alignment to the *Homo sapiens* hg19 reference sequence was performed with the Torrent Suite™ v4.6 with—g 0 parameter for the alignment step with tmap. Genome binning was performed using WindowMaker tool from BEDTOOLS suite²⁻⁴. Read counting and assignment to genomic bins were performed using the HTSeq library²⁻⁴. Reads spanning more than one bin were assigned to the one with the longest overlap. Read counting and assignment to MseI fragments were performed by BEDTOOLS IntersectBed tool, filtering out reads with more than one fragment match. GC-based normalization was performed by LOWESS fitting of per-bin GC content versus read count on each bin. Calculation of bin mappability value was performed using bigWigAverageOverBed (<http://hgdownload.cse.ucsc.edu/admin/exe/>) using mappability track for 100mers produced by Encode/CRG (wgEncodeCrgMapabilityAlign100mer; downloaded from <https://genome.ucsc.edu/>).

Copy Number Alteration (CNA) calling: Control-FREEC (Control-Free Copy number caller) software was used to obtain copy-number calls, using the mode without control sample [3]. Read counts were corrected by GC content and mappability (uniqMatch option). Bin size was manually set in order to match the desired resolution. To determine significant CNA calls, Wilcoxon test and Kolmogorov-Smirnov test (p value < 0.01) were performed using the script to assess significance²⁻⁴. Supplementary Table 1 lists the individual cancer genes interrogated.

CTC CNV Assay	
Accuracy	89.2%
Precision	85% (CV<15%)
Specificity	100%
Sensitivity	82.3%
Positive Predictive Value	100%
Limit of detection	Single cell

Purification of cfDNA and Extraction of CTCs: Blood samples were fractionated within 96 hours of fixation, by centrifugation (500xg, 10min). The upper layer of plasma was collected and cfDNA isolated using Qiagen's Circulating Nucleic Acid kit per manufacturer's instructions. The remaining blood cell fraction was then processed as described previously^{5,6}. Briefly, initial incubation with biotinylated antibodies against EpCAM, TROP2, HER2, and PSMA (Cynvenio Biosystems) was followed by incubation with streptavidin magnetic particles. The pre-processed blood was then loaded onto the LiquidBiopsy® platform which performs automated immunomagnetic enrichment and immunofluorescent staining on a microfluidic flow cell. Cells were stained with DAPI and fluorescent antibodies targeting cytokeratin, CD45, PDL1 (Cynvenio Biosystems). Cells captured in the flow cell were imaged and enumerated using the Ariol system (Leica Biosystems). Following image analysis, cells were eluted from the microfluidic device and processed for sequencing. CTC samples are processed for sequencing irrespective of enumeration results. CTC-DNA extraction was performed by re-suspending the eluted

cell pellet in 6.5 μ L LB Digest containing Proteinase K followed by incubation at 55°C for 3 hours, then 70°C for 1 hour as reported previously ⁵. The whole cell lysate was then used for a multiplex PCR reaction with Cynvenio's 27-gene ClearID panel (Supplementary Table 2) followed by clean-up using AMPureXP beads.

Sequencing analysis for CTC-DNA and cfDNA: Sequencing libraries for cell-free DNA, CTC, and germline DNA were then prepared as described previously using Cynvenio's 27-gene panel in Supplementary Table 2 ^{5,6}. Libraries were quantified using the Taqman Library Quantification Kit (Life Technologies) and an ViiA7 qPCR machine (Applied Biosystems). Germline, CTC-DNA and cfDNA libraries were the sequenced on an Ion Torrent S5XL sequencer. Single-nucleotide variants were called using Everest Software (Cynvenio), which identifies variants in CTC- and cf-DNA that are not present in germline samples (case-control sequencing). Variants expressed at $\geq 1\%$ allelic frequency are reported. Only amplicons with greater than 2000 reads are reported. At $\sim 1\%$ detection threshold, this requires 20 reads to be informative.

CTC DNA SSNV Assay		cfDNA SSNV Assay	
Accuracy	95%	Accuracy	100%
Precision	85% (CV<15%)	Precision	95%
Linearity	R ² =0.968	Linearity	R ² =0.9967
Reportable Range	9-90 CTC cell/mL	Lower limit of detection	1.1%

RNA Extraction, RT-PCR, relative cfRNA expression

Fractionation of plasma and extraction of cfRNA: Whole blood (7.5 ml) was collected in cfRNA Streck tubes and fractionated by centrifugation (300xg, 20 min) to isolate the upper plasma layer. The plasma layer was centrifuged (5000 xg, 10 min). cfRNA was extracted from 2mL of plasma with a proprietary in-house developed protocol ⁷. All nucleic acids were kept in bar-coded matrix storage tubes. RNA was stored either at -80°C or at +4°C as complementary DNA (cDNA) via random-primed reverse-transcription.

The detection of *AR* and *ARV7* with quantitative real-time PCR: Expression of *AR* and *ARV7* were measured by quantitative real-time PCR to detect their expression from cfRNA using appropriate gene-specific primers. Amplification was performed using a proprietary method (Liquid Genomics) in a 10 μ L reaction mix containing 2 μ L cDNA, the primer and probe ⁷. β -actin was used as an internal control. Delta Ct (dCT) was calculated from the Ct value using β -actin as the internal control. dCT was the Ct value of *AR* and *ARV7* subtracted by the Ct value of β -actin. K was calculated to produce whole number for relative gene expression control using RNA isolated from universal human reference RNA (*UHR*) as a positive control for *AR* and an *ARV7* synthetic fragment spiked into *UHR* as a positive control for *ARV7*. Using this value of K, relative gene expression = $(2^{-dCT}) * K$.

cfRNA Relative Expression Assay

Accuracy	90%
Overall Precision	99%
Limit of Detection	13 copies
Repeatability	100%

SUPPLEMENTARY REFERENCES

1. Goldkorn A, Ely B, Quinn DI, et al. Circulating tumor cell counts are prognostic of overall survival in SWOG S0421: a phase III trial of docetaxel with or without atrasentan for metastatic castration-resistant prostate cancer. *J Clin Oncol*. 2014;32(11):1136-1142.
2. Quinlan AR, Clark RA, Sokolova S, et al. Genome-wide mapping and assembly of structural variant breakpoints in the mouse genome. *Genome Res*. 2010;20(5):623-635.
3. Anders S, Pyl PT, Huber W. HTSeq--a Python framework to work with high-throughput sequencing data. *Bioinformatics*. 2015;31(2):166-169.
4. Boeva V, Popova T, Bleakley K, et al. Control-FREEC: a tool for assessing copy number and allelic content using next-generation sequencing data. *Bioinformatics*. 2012;28(3):423-425.
5. Winer-Jones JP, Vahidi B, Arquilevich N, et al. Circulating tumor cells: clinically relevant molecular access based on a novel CTC flow cell. *PLoS One*. 2014;9(1):e86717.
6. Strauss WM, Carter C, Simmons J, et al. Analysis of tumor template from multiple compartments in a blood sample provides complementary access to peripheral tumor biomarkers. *Oncotarget*. 2016;7(18):26724-26738.
7. Ishiba T, Hoffmann AC, Usher J, et al. Frequencies and expression levels of programmed death ligand 1 (PD-L1) in circulating tumor RNA (ctRNA) in various cancer types. *Biochem Biophys Res Commun*. 2018;500(3):621-625.

SUPPLEMENTARY TABLES

ID	Age	Mets			Last PSA at Blood Draw	CRPC Treatment History					Tx at Blood Draw (R= Responding, P=Progressing)	Solid Tumor NGS		
		LN	Bone	Visceral		Abi	Enza	SipT	DTX	Other		Tissue Site (Time between bx and blood draw in mos)	# SSNVs	# CNVs (amps/dels)
1	60		+	Liver	52.05				+		DTX (R)			
2	76	+	+	Liver	T1: 2.49 T2: 6.27	+				Radium-223 PARP-I Carboplatin	T1: PARP-I (R) T2: Carboplatin (P)	Liver Met (1)	2	Amp: 7 Del: 0
3	76	+	+	Brain	59.63	+	+	+	+	Radium-223	Radium-223 (R)	LN (0)	2	Amp: 9 Del: 0
4	61	+	+	Liver	269.1	+	+	+	+	Cabazitaxel PARP-I	PARP-I (P)	LN (5)	1	Amp: 1 Del: 0
5	72	+	+	Lung, Spleen	194.7	+		+	+	Dasatanib PARP-I	PARP-I (R)	LN (0)	2	Amp: 2 Del: 1
6	77		+	Liver	326.2	+	+			Enza + Abi	None (P)			
7	59		+		11.2		+	+	+	Caboxantinib	Caboxantinib (R)	Prostate (15)		Amp: 8 Del: 0
8	63		+	Lung	144.2	+	+		+		DTX (R)	Bone Met (18)	3	Amp: 0 Del: 0
9	81	+	+	Lung	T1: 44.22 T2: 14.71	+			+	Radium-223	T1: DTX (R) T2: DTX (P)			
10	46	+	+		T1: 55.18 T2: 434.6	+				Radium-223 Pembro PARP-I Caboxantinib	T1: PARP-I (R) T2: Caboxantinib (R)	Prostate (38)	1	Amp: 0 Del: 0
11	69	+	+		0	+	+	+			Enza + Abi (R)			
12	73			Lung	T1: 26.06, T2: 7.76		+	+			T1: SipT + Enza (R) T2: Enza (R)			
13	69		+		89.00	+	+		+	Radium-223	Radium-223 + Enza (R)			
14	79	+	+		T1: 0.61 T2: 1.2		+	+			T1: Enza (R) T2: Enza (R)			
15	79	+	+	Brain, Liver	T1: 17.52 T2: 1.07				+	Pembro Carboplatin Fluorouracil	T1: None (P) T2: Fluorouracil (R)	LN (2)	1	Amp: 0 Del: 1
16	57		+	Liver, Lung	425						None (P)			
17	71		+		6.90	+	+	+		Radium-223	Radium-223 + Enza (R)			
18	59	+	+	Liver, Lung	T1: 203 T2: 261	+	+		+	Cabazitaxel	T1: Cabazitaxel (R) T2: MXT (R)			
19	79		+		44.22	+	+		+		Abi (R)	Prostate (48)	1	Amp: 0 Del: 2
20	65	+	+		T1: 81.86 T2: 103.4	+	+				T1: Abi (R) T2: Enza (R)	Bone Met (0)	0	Amp: 2 Del: 1

Supplementary Table 1. Summary chart of patient clinical data. T1 = first blood draw, T2= second blood draw. For treatment status, P= progressing on treatment, R= responding to treatment. Abi is abiraterone, Enza is enzalutamide, SipT is sipuleucel-T, and DTX is docetaxel.

ID	CTC #	CTC CNV#	CTC DNA SSNVs	cfDNA SSNVs	cfRNA ARV7 Expression (ARV7/AR Ratio)
1	421	NA	<i>TP53_G199*</i> <i>TP53_R196*</i>	<i>TP53_R196*</i>	ND
2	T1: 401 T2: 692	T1: 29/2 T2: NA	T1: ND T2: ND	T1: ND T2: ND	T1: ND T2: ND
3	168	25/3	<i>TP53_Q104*</i>	ND	ND
4	50	22/16	<i>TP53_S241C</i>	<i>PIK3CA_E545A</i> <i>TP53_S241C</i>	0.4 (0.016)
5	42	NA	ND	<i>PIK3CA_E545A</i> <i>TP53_R213*</i>	1.84 (0.005)
6	17	NA	NA	NA	ND
7	9	26/9	ND	ND	ND
8	8	NA	NA	ND	ND
9	T1: 6 T2: 2	NA	T1: <i>HRAS_G12S</i> T2: ND	T1: <i>PIK3CA_E542K</i> T2: <i>TP53_G105C</i>	T1: ND T2: ND
10	T1: 3 T2: 11	NA	T1: <i>TP53_H193Y</i> T2: <i>TP53_H193Y</i>	T1: <i>TP53_H193Y</i> T2: <i>TP53_H193Y</i> , <i>TP53_E56*</i>	T1: ND T2: 31.19 (0.159)
11	0	NA	ND	<i>TP53_C141Y</i>	ND
12	T1: 0 T2: 0	NA	T1: ND T2: ND	T1: <i>EGFR_T790M</i> T2: <i>EGFR_S720F</i>	T1: ND T2: ND
13	0	NA	ND	<i>HRAS_G12S</i>	ND
14	T1: 0 T2: 0	NA	T1: NA T2: ND	T1: <i>TP53_R282W</i> T2: <i>TP53_R158H</i> , <i>PIK3CA_E545A</i>	T1: ND T2: ND
15	T1: 234 T2: 560	T1: 33/39 T2: 9/13	T1: ND T2: ND	T1: NA T2: ND	T1: ND T2: ND
16	1	NA	<i>TP53_R280G</i>	<i>PIK3CA_E545K</i> , <i>TP53_V272L</i> , <i>TP53_Q144R</i>	ND
17	0	NA	ND	<i>PIK3CA_H1047Y</i>	ND
18	T1: 1 T2: NA	NA	T1: ND T2: <i>TP53_R213*</i>	T1: <i>TP53_R213*</i> T2: ND	T1: ND T2: 43.23 (0.062)
19	16	NA	ND	ND	ND
20	T1: 31 T2: 22	T1: 27/15 T2: NA	T1: ND T2: <i>ATM_R337C</i>	T1: ND T2: <i>CKN2A_A68T</i>	T1: 47.09 (0.116) T2: 48.80 (0.332)

Supplementary Table 2. Summary chart of Liquid Biopsy Alterations. T1 = first blood draw, T2= second blood draw. For CTC CNV, two numbers separated by “/” denote the number of CNVs detected in each individual CTC. ND= none detected, NA= not available (assay could not be performed due to platform failure or sample not meeting eligibility criteria (e.g. minimum CTC # or purity)).

<i>EPCAM</i>	<i>BCL6</i>	<i>CHD1</i>	<i>IKZF1</i>	<i>NBN</i>	<i>FAS</i>	<i>FGF4</i>	<i>FOXA1</i>	<i>ERBB2</i>	<i>TMPRSS2</i>
<i>ERBB4</i>	<i>FANCD2</i>	<i>MAP3K1</i>	<i>PMS2</i>	<i>PREX2</i>	<i>PTEN</i>	<i>HRAS</i>	<i>RAD51B</i>	<i>TP53</i>	<i>CHEK2</i>
<i>MSH2</i>	<i>MLH1</i>	<i>ESR1</i>	<i>GRHL2</i>	<i>PRKDC</i>	<i>ATM</i>	<i>MDM2</i>	<i>FANCI</i>	<i>CCNE1</i>	<i>AR</i>
<i>MSH6</i>	<i>PIK3CA</i>	<i>BRAF</i>	<i>LYN</i>	<i>RUNX1T1</i>	<i>CCND1</i>	<i>BRCA2</i>	<i>FANCA</i>	<i>KLK2</i>	<i>MED12</i>
<i>PDK1</i>	<i>SOX2</i>	<i>EGFR</i>	<i>MYC</i>	<i>PCA3</i>	<i>FGF19</i>	<i>KLF5</i>	<i>PALB2</i>	<i>KLK3</i>	
<i>STAT4</i>	<i>TERC</i>	<i>EZH2</i>	<i>MYST3</i>	<i>PD-L1</i>	<i>FGF3</i>	<i>RB1</i>	<i>BRCA1</i>	<i>RUNX1</i>	

Supplementary Table 3. Gene Panel for CNV analysis

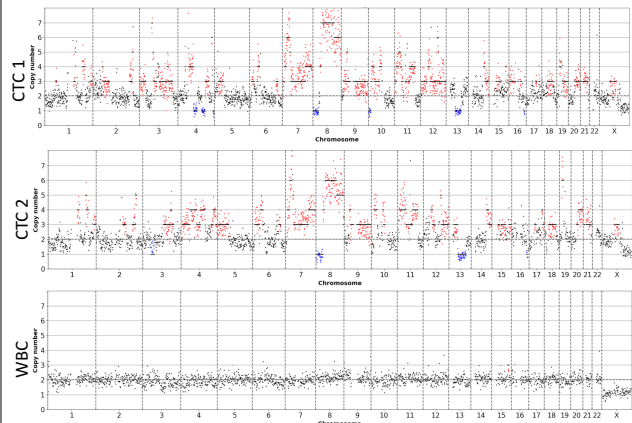
<i>AKT1</i>	<i>CHD1</i>	<i>ESR1</i>	<i>KRAS</i>	<i>MAP3K1</i>	<i>PIK3CA</i>	<i>RYR2</i>
<i>ARID1A</i>	<i>CDKN2A</i>	<i>GATA3</i>	<i>LRP1</i>	<i>MED12</i>	<i>PTEN</i>	<i>RYR3</i>
<i>ATM</i>	<i>EGFR</i>	<i>HRAS</i>	<i>LRP2</i>	<i>NCOR1</i>	<i>RB1</i>	<i>TP53</i>
<i>BRAF</i>	<i>ERBB2</i>	<i>KMT2C</i>	<i>MAP2K4</i>	<i>NRAS</i>	<i>RUNX1</i>	

Supplementary Table 4. Gene panel for SSNV analysis.

SUPPLEMENTARY DATA

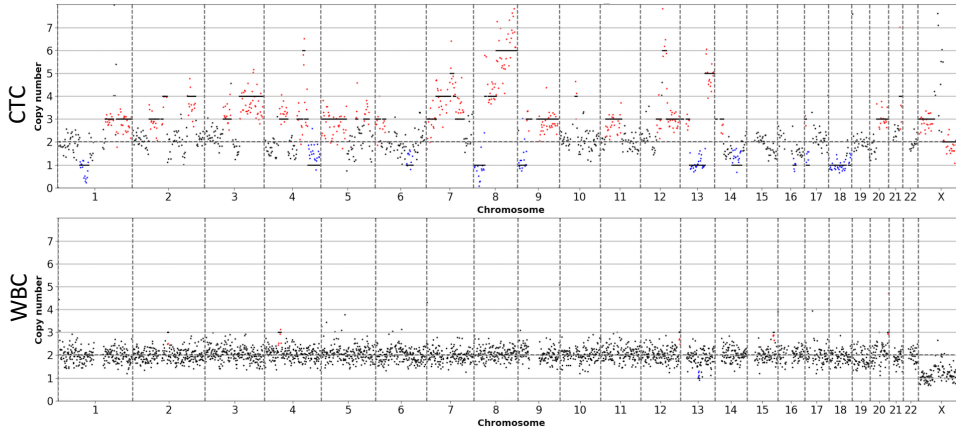
Supplementary Data: Figure 1(A-E). CTC CNV Summary Data for Patients 2, 3, 4, 7, 15-T2. Chromosome spread with amplifications (red) and losses (blue) for individual CTCs and control germline WBC. Table outlines CNVs for genes queried in our panel (Supplementary Table 1) in solid biopsy, CTCs, and WBC.

Patient 2 CNV Data



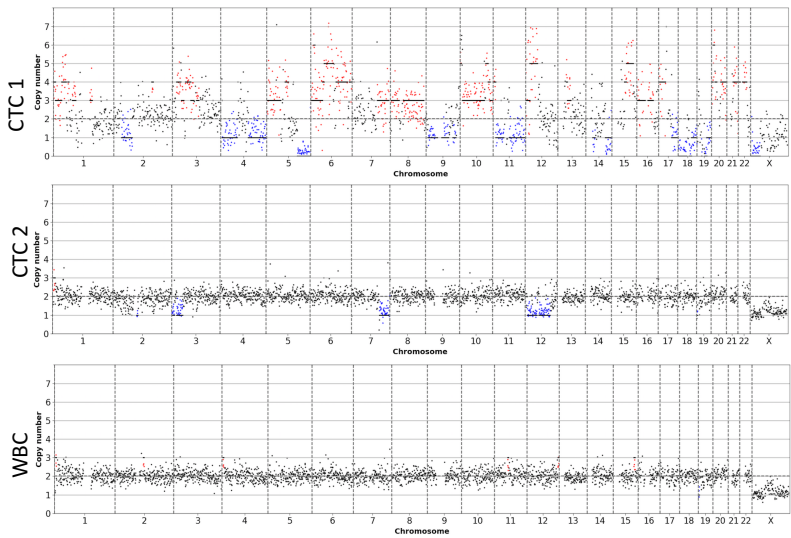
Gene	Chrom #	Status in Liver Met	CNV Status in CTC 1	Copy Number in CTC 1	CNV Status in CTC 2	Copy Number in CTC 2	CNV Status in WBC	Copy Number in WBC
STAT4	2	Normal	Gain	3	Gain	3	Normal	2
FANCD2	3	Normal	Gain	3	Normal	2	Normal	2
TERC	3	Normal	Gain	3	Normal	2	Normal	2
MAP3K1	5	Normal	Normal	2	Gain	3	Normal	2
BRAF	7	Normal	Gain	4	Gain	4	Normal	2
EGFR	7	Normal	Gain	3	Gain	3	Normal	2
EZH2	7	Normal	Gain	4	Gain	4	Normal	2
IKZF1	7	Normal	Gain	3	Gain	3	Normal	2
PMS2	7	Normal	Gain	3	Normal	2	Normal	2
GRHL2	8	NA	Gain	7	Gain	6	Normal	2
LYN	8	Amplification	Gain	7	Gain	6	Normal	2
MYC	8	Normal	Gain	6	Gain	5	Normal	2
MYST3	8	Amplification	Gain	7	Gain	4	Normal	2
NBN	8	NA	Gain	7	Gain	6	Gain	3
PREX2	8	Amplification	Gain	7	Gain	6	Normal	2
PRKDC	8	Amplification	Gain	7	Gain	6	Normal	2
RUNX1T1	8	Amplification	Gain	7	Gain	6	Normal	2
PCA3	9	NA	Gain	3	Gain	3	Normal	2
FAS	10	Loss	Normal	2	Normal	2	Normal	2
ATM	11	ATM_Q1017* ATM_F2181D	Normal	2	Normal	2	Normal	2
CCND1	11	Amplification	Gain	18	Gain	19	Normal	2
FGF19	11	Amplification	Gain	18	Gain	19	Normal	2
FGF3	11	Normal	Gain	18	Gain	19	Normal	2
FGF4	11	Rearrangement	Gain	18	Gain	19	Normal	2
MDM2	12	Normal	Gain	3	Normal	2	Normal	2
KLF5	13	NA	Loss	1	Loss	1	Normal	2
RB1	13	Normal	Loss	1	Loss	1	Normal	2
FANCI	15	NA	Normal	2	Gain	3	Gain	3
PALB2	16	Normal	Gain	3	Normal	2	Normal	2
KLK2	19	NA	Gain	3	Normal	2	Normal	2
KLK3	19	NA	Gain	3	Normal	2	Normal	2
RUNX1	21	Normal	Gain	3	Gain	3	Normal	2
TMPRSS2	21	TMPRSS2-ERG Fusion	Normal	2	Normal	2	Normal	2
AR	X	Normal	Normal	1	Gain	3	Normal	1
MED12	X	Normal	Normal	1	Normal	1	Normal	1

Patient 3 CNV Data



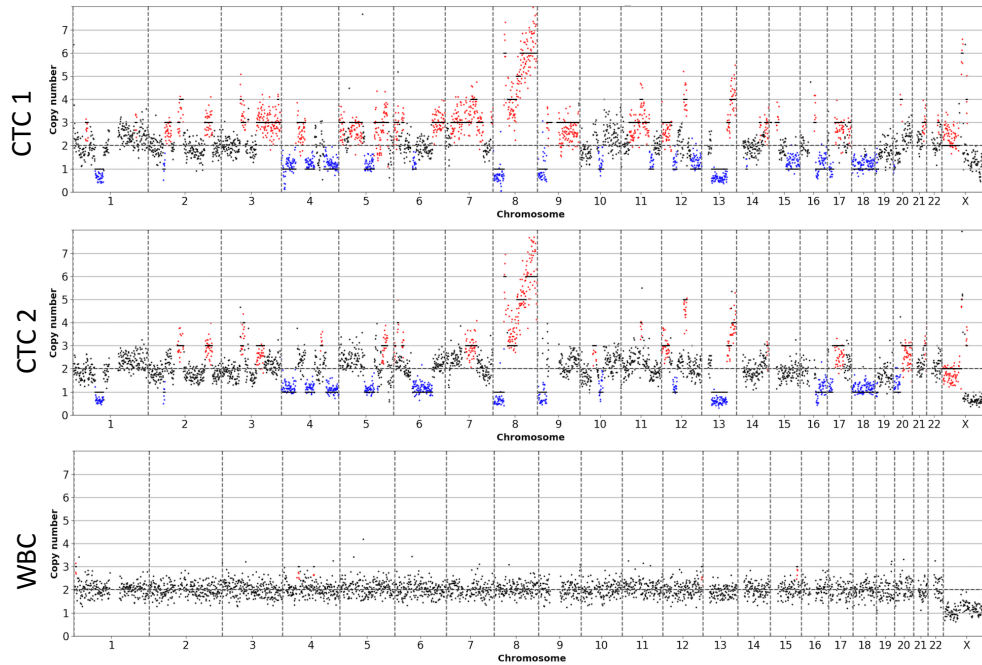
Gene	Chrom #	Status in LN	CNV Status in CTC	Copy Number in CTC	CNV Status in WBC	Copy Number in WBC
ERBB4	2	Amplification	Normal	2	Normal	2
PDK1	2	Amplification Rearrangement	Loss	1	Normal	2
STAT4	2	Amplification	Gain	4	Normal	2
BCL6	3	Amplification	Gain	4	Normal	2
PIK3CA	3	Normal	Gain	4	Normal	2
SOX2	3	Amplification	Gain	4	Normal	2
TERC	3	Amplification	Gain	4	Normal	2
MAP3K1	5	Normal	Gain	3	No call	NA
EGFR	7	Normal	Gain	4	Normal	2
IKZF1	7	Normal	Gain	4	Normal	2
GRHL2	8	NA	Gain	6	Normal	2
LYN	8	Normal	Gain	4	Normal	2
MYC	8	Amplification	Gain	6	Normal	2
MYST3	8	Normal	Gain	4	Normal	2
NBN	8	NA	Gain	6	Normal	2
PREX2	8	Normal	Gain	4	Normal	2
PRKDC	8	Normal	Gain	4	Normal	2
RUNX1T1	8	Normal	Gain	6	Normal	2
PCA3	9	NA	Gain	3	Normal	2
CCND1	11	Amplification	Gain	3	Normal	2
FGF19	11	Normal	Gain	3	Normal	2
FGF3	11	Normal	Gain	3	Normal	2
FGF4	11	Normal	Gain	3	Normal	2
MDM2	12	Normal	Gain	3	Normal	2
BRCA2	13	Normal	Loss	1	Normal	2
KLF5	13	NA	Loss	1	Normal	2
RB1	13	Normal	Loss	1	Normal	2
RAD51B	14	NA	Loss	1	Normal	2
TP53	17	Missense Mutation	Loss	1	Normal	2
TMPRSS2	21	Normal	Gain	4	Normal	2
AR	X	Normal	Gain	7	Normal	1
MED12	X	Normal	Gain	3	No Call	NA

Patient 4 CNV Data



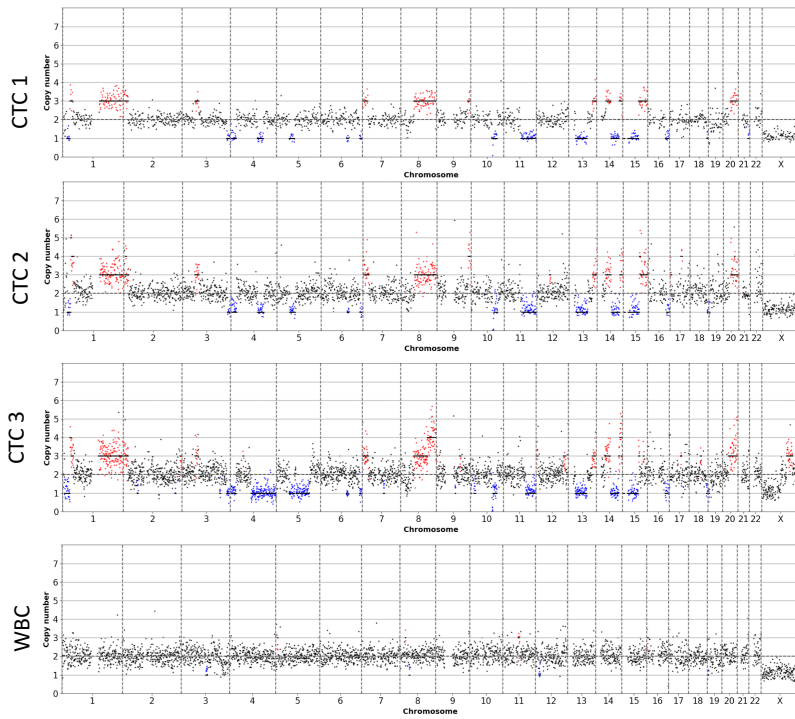
Gene	Chrom #	Status in LN	CNV Status in CTC 1	Copy Number in CTC 1	CNV Status in CTC 2	Copy Number in CTC 2	CNV Status in WBC	Copy Number in WBC
EPCAM	2	NA	Loss	1	Normal	2	Normal	2
MSH2	2	Normal	Loss	1	Normal	2	Normal	2
MSH6	2	Normal	Loss	1	Normal	2	Normal	2
FANCD2	3	Normal	Gain	3	Gain	3	Normal	2
MLH1	3	Normal	Gain	3	Loss	1	Normal	2
MAP3K1	5	Normal	Gain	3	No Call	NA	Normal	2
ESR1	6	Normal	Gain	3	Normal	2	Normal	2
BRAF	7	Normal	Gain	3	Loss	1	Normal	2
EZH2	7	Normal	Gain	3	Loss	1	Normal	2
GRHL2	8	NA	Gain	3	Normal	2	Normal	2
LYN	8	Normal	Gain	3	Normal	2	Normal	2
MYC	8	Normal	Gain	3	Normal	2	Normal	2
NBN	8	NA	Gain	3	Normal	2	Normal	2
PREX2	8	Normal	Gain	3	Normal	2	Normal	2
RUNX1T1	8	Normal	Gain	3	Normal	2	Normal	2
PCA3	9	Normal	Loss	1	Normal	2	Normal	2
FAS	10	Normal	Gain	3	Normal	2	Normal	2
PTEN	10	Frameshift Mutation	Gain	3	Normal	2	Normal	2
ATM	11	Normal	Loss	1	Normal	2	Normal	2
CCND1	11	Normal	Loss	1	Normal	2	Normal	2
FGF19	11	Normal	Loss	1	Normal	2	Normal	2
FGF3	11	Normal	Loss	1	Normal	2	Normal	2
FGF4	11	Normal	Loss	1	Normal	2	Normal	2
MDM2	12	Normal	Normal	2	Loss	1	Normal	2
BRCA2	13	Normal	Gain	3	Normal	2	Normal	2
RB1	13	Normal	Gain	3	Normal	2	Normal	2
FOXA1	14	NA	Loss	1	Normal	2	Normal	2
FANCI	15	NA	Gain	3	Normal	2	Gain	3
PALB2	16	Normal	Gain	3	Normal	2	Normal	2
TP53	17	Point Mutation	Gain	4	Normal	2	Normal	2
KLK2	19	NA	Loss	1	Normal	2	Normal	2
KLK3	19	NA	Loss	1	Normal	2	Normal	2
RUNX1	21	Normal	Gain	4	Normal	2	Normal	2
TMPRSS2	21	Normal	Gain	4	Normal	2	Normal	2
CHEK2	22	Normal	Gain	4	Normal	2	Normal	2
AR	X	Amplification	Normal	1	Normal	1	Normal	1

Patient 7 CNV Data



GeneN	Chrom #	Status in Prostate	CNV Status in CTC 1	Copy Number in CTC 1	CNV Status in CTC 2	Copy Number in CTC 2	CNV Status in WBC	Copy Number in WBC
STAT4	2	Normal	Gain	3	Gain	3	Normal	2
BCL6	3	Normal	Loss	1	Normal	2	Normal	2
PIK3CA	3	Normal	Gain	3	Normal	2	Normal	2
SOX2	3	Normal	Gain	3	Normal	2	Normal	2
TERC	3	Normal	Gain	3	Normal	2	Normal	2
CHD1	5	Normal	Loss	1	Loss	1	Normal	2
MAP3K1	5	Normal	Gain	3	Normal	2	Normal	2
ESR1	6	Normal	Gain	3	Normal	2	Normal	2
EGFR	7	Normal	Gain	3	Normal	2	Normal	2
IKZF1	7	Amplification	Gain	3	Normal	2	Normal	2
GRHL2	8	NA	Gain	6	Gain	5	Normal	2
LYN	8	Normal	Gain	4	Gain	3	Normal	2
MYC	8	Amplification	Gain	6	Gain	6	Normal	2
MYST3	8	Normal	Gain	4	Gain	3	Normal	2
NBN	8	NA	Gain	6	Gain	5	Normal	2
PREX2	8	Normal	Gain	4	Gain	3	Normal	2
PRKDC	8	Normal	Gain	4	Gain	3	Normal	2
RUNX1T1	8	Normal	Gain	6	Gain	5	Normal	2
PCA3	9	NA	Gain	3	Normal	2	Normal	2
PD-L1	9	Normal	Loss	1	Loss	1	Normal	2
ATM	11	Normal	Loss	1	Normal	2	Normal	2
CCND1	11	Amplification	Gain	4	Gain	4	Normal	2
FGF19	11	Amplification	Gain	4	Gain	4	Normal	2
FGF3	11	Amplification	Gain	4	Gain	4	Normal	2
FGF4	11	Amplification	Gain	4	Gain	4	Normal	2
MDM2	12	Amplification	Normal	2	Normal	2	Normal	2
BRCA2	13	Normal	Loss	1	Loss	1	Normal	2
KLF5	13	NA	Loss	1	Loss	1	Normal	2
RB1	13	Normal	Loss	1	Loss	1	Normal	2
FANCI	15	NA	Loss	1	Normal	2	Gain	3
BRCA1	17	Normal	Gain	3	Gain	3	Normal	2
ERBB2	17	Normal	Gain	3	Gain	3	Normal	2
TP53	17	Normal	Loss	1	Loss	1	Normal	2
RUNX1	21	Normal	Gain	3	Normal	2	Normal	2
TMPRSS2	21	Normal	Gain	3	Gain	3	Normal	2
AR	X	Amplification Rearrangement	Gain	6	Gain	5	Normal	1
MED12	X	Normal	Normal	1	Normal	1	Normal	1

Patient 15-T2



Gene	Chrom #	Status in LN	CNV Status in CTC 1	Copy Number in CTC 1	CNV Status in CTC 2	Copy Number in CTC 2	CNV Status in CTC 3	Copy Number in CTC 3	CNV Status in WBC	Copy Number in WBC
BCL6	3	Normal	Loss	1	Loss	1	Loss	1	Normal	2
CHD1	5	NA	Normal	2	Normal	2	Loss	1	No Call	NA
MAP3K1	5	Normal	Loss	1	Loss	1	Loss	1	Normal	2
PMS2	7	Normal	No Call	NA	Gain	3	No Call	NA	Normal	2
GRHL2	8	NA	Gain	3	Gain	3	Gain	3	Normal	2
LYN	8	Normal	Gain	3	Gain	3	Gain	3	Normal	2
MYC	8	Normal	Gain	3	Gain	3	Gain	4	Normal	2
NBN	8	NA	Gain	3	Gain	3	Gain	3	Normal	2
PREX2	8	Normal	Gain	3	Gain	3	Gain	3	Normal	2
RUNX1T1	8	Normal	Normal	2	Gain	3	Gain	3	Normal	2
PCA3	9	NA	Normal	2	Normal	2	Loss	1	Normal	2
FAS	10	Loss	Loss	1	Loss	0	Loss	0	Normal	2
PTEN	10	Loss	No Call	NA	No Call	NA	Loss	0	No Call	NA
ATM	11	Normal	Loss	1	Loss	1	Loss	1	Normal	2
CCND1	11	Normal	Normal	2	Normal	2	Normal	2	Gain	3
FGF19	11	Normal	Normal	2	Normal	2	Normal	2	Gain	3
FGF3	11	Normal	Normal	2	Normal	2	Normal	2	Gain	3
FGF4	11	Normal	Normal	2	Normal	2	Normal	2	Gain	3
BRCA2	13	Nonsense Mutation	Loss	1	Loss	1	Loss	1	Normal	2
KLF5	13	NA	Loss	1	Loss	1	Loss	1	Normal	2
RB1	13	Normal	Loss	1	Loss	1	Loss	1	Normal	2
FOXA1	14	NA	Gain	3	Gain	3	Normal	2	Normal	2
RAD51B	14	NA	Loss	1	Loss	1	Loss	1	Normal	2
FANCI	15	NA	Gain	3	Gain	3	Normal	2	Normal	2
FANCA	16	Normal	Loss	1	Loss	1	Loss	1	Loss	1
ERBB2	17	Missense Mutation	Normal	2	Normal	2	Normal	2	Normal	2
TMPRSS2	21	TMPRSS2-ERG Fusion	Loss	1	Normal	2	Normal	2	Normal	2